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ORIGINAL ARTICLE

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Diagnostic Accuracy of the Allergy Lateral Flow Assay (ALFA) Specific Allergen Test to *Dermatophagoides pteronyssinus* in Children Ages 8 to 18 years old Diagnosed with Allergic Rhinitis Seen at the Philippine General Hospital, Allergy Clinic

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ABSTRACT

Objectives: Allergic rhinitis is the most common chronic disorder in the pediatric population. The hallmark of an allergic condition is the evidence of specific IgE to an allergen. The ALFA Specific Allergen Test is a rapid *in vitro* assay for specific IgE. This study aims to determine the diagnostic accuracy of the ALFA Allergy System in diagnosing children aged 8 to 18 years old with allergic rhinitis.

Methodology: This is a prospective study of diagnostic test accuracy performed at the Philippine General Hospital, Allergy Clinic. The skin prick test and the ALFA Specific Allergen Test to *Dermatophagoides pteronyssinus* were performed on 77 pediatric patients aged 8 to 18 years old with allergic rhinitis from September to February 2017.

Results: The ALFA Specific Allergen Test to *Dermatophagoides pteronyssinus* had a sensitivity of 84.8%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 81.6%. The positive likelihood ratio could not be computed since the specificity was 100%; the negative likelihood ratio was 0.15. The area under the Receiver Operating Characteristics (ROC) curve was 0.924.

Conclusions: Based on the results, the ALFA Specific Allergen Test is a reliable test in determining the presence and absence of specific IgE to *Dermatophagoides pteronyssinus*.

Keywords: allergic rhinitis, skin test, *in vitro* assay, ALFA Specific Allergen Test, *Dermatophagoides*

INTRODUCTION

Allergic rhinitis is an inflammatory disorder of the nasal mucosa initiated by an allergic immune response to inhaled allergens in a sensitized individual. It is an allergic disease that is caused by hypersensitivity to airborne allergens. Aside from symptoms related to allergic rhinitis, such as rhinorrhea, nasal congestion, sneezing, and nasal pruritus, patients often complain of fatigue, irritability, frustration, self-consciousness, and a decreased ability to have energy, motivation, alertness, and concentrate.[1] It is a major chronic disease of children based on its high prevalence, co-morbidities, and detrimental effects on the quality of life and school performance.

Allergic rhinitis is presently the most common allergic condition affecting individuals of both genders and all ages. It is the most common chronic disorder in the pediatric population, with up to 40% of children affected.[2] High prevalence is recorded in the developed nations of the Northern Hemisphere, with 20-30% of the affected population in Europe and 12-30% in the United States. In Southeast Asia, 5.5-44.2% are affected by allergic rhinitis. The International Study of Asthma and Allergies in Childhood (ISAAC) program, comprising phases I, II, and III, conducted at more than 237 centers in 98 countries worldwide, has demonstrated significant global variations in the prevalence of allergic rhinitis in children. Some of the highest increases were observed in the non-Western region, particularly in the Asia-Pacific, compared to the Western population, such as North America. Based on the ISAAC Phase I study, which involved the Philippines, 15.3% of children aged 13-14 years old had allergic rhinitis; in the ISAAC Phase III study, the prevalence was 11.1% among the same age group.[4] Allergic rhinitis was more common in women at 55%. There is a high prevalence in childhood, but with age, its prevalence decreases.[5] In a local study done by Abong JM et al., the prevalence of allergic rhinitis among Filipino adults in 2008 was 20%.[1]

Persistent nasal dysfunction may result in significant decreases in physical and emotional functioning, energy, general health perception, and social functioning, leading to absences from school and work, reduced worker productivity, and impaired school performance. In addition, chronic nasal inflammation may aggravate or lead to the development of other significant disorders, including asthma, rhinosinusitis, and middle ear diseases.[2]

The hallmark of any true allergic condition, such as allergic rhinitis, is evidence of specific IgE to a relevant allergen in the affected individual. Determination of specific IgE, preferably by skin prick testing, is indicated to provide evidence of an allergic basis for the patient's symptoms, to confirm or exclude suspected causes of the patient's symptoms, or to assess the sensitivity to a specific allergen for avoidance measures and/or allergen immunotherapy.[6] Skin prick tests are widely used to demonstrate an immediate IgE-mediated allergic reaction. They represent a primary diagnostic tool in the field of allergy.[7] Prick/puncture tests confirm clinical sensitivity induced by aeroallergens, food, some drugs, and a few chemicals. The diagnostic accuracy of the prick/puncture test for aeroallergens has also been confirmed in clinically allergic patients undergoing specific nasal bronchoprovocation challenge. The prick/puncture test showed a sensitivity of 85% to 87%, while the specificity of this test ranged from 79% to 86%.[8] Positive likelihood ratios for cat, tree pollen, grass pollen, and house dust allergens showed values of 4.93, 16.17, 3.23, and 4.06, respectively, with corresponding negative likelihood ratios of 0.08, 0.03, 0.04, and 0.03.[8] Skin prick testing is generally recommended as the first choice in the diagnostic workup for allergic diseases. According to the updated practice parameter on the diagnosis and management of rhinitis developed by the Joint Task Force on Practice Parameters of the American Academy of Allergy, Asthma and Immunology (AAAAI), American College of Asthma, Allergy and Immunology (ACAAI) and the Joint Council of Allergy, Asthma and Immunology, skin prick tests are the preferred tests for the diagnosis of IgE mediated sensitivity. The number of skin prick tests and the allergens selected for skin testing should be determined based on the patient's age, history, environment, and living conditions, such as the area of the country, occupation, and activities.[5] The skin prick test is the most common screening method for allergy evaluation, which can provide proper confirmatory evidence for the diagnosis of specific allergies, as stated in the task force on allergy testing developed by the Philippine Society of Asthma, Allergy and Immunology.[9] Their characteristics of simplicity, rapidity of performance, low cost, and high sensitivity define their key position in allergy diagnosis.[10] The reliability of prick/puncture tests depends on the tester's skill, the test instrument, the skin color, skin reactivity on the day of the test, potency, and stability of test reagents.[11] However, skin prick testing is challenging in patients with severe eczema, dermatographism, or those taking antihistamines or other medications that can interfere with the proper interpretation of test results. Also, there is always a small risk of an anaphylactic reaction (<0.02%).[12]

Due to some drawbacks associated with skin prick testing, rapid *in vitro* assays for specific IgE detection have been developed as a promising alternative for point-of-care diagnostics. The ALFA (Allergy Lateral Flow Assay) Specific Allergen Test combines the advantages of lateral flow devices with the flexibility of choosing different allergens. It is specifically designed for use in a doctor's office. It is the first test to be launched as a simple blood spot test that detects specific IgE antibodies.[11]

The objective of this study is to evaluate the sensitivity and specificity of the ALFA Specific Allergen Test compared to the skin prick testing in the detection of specific IgE to a species of house dust mite, *Dermatophagoides pteronyssinus*, in children ages 8 to 18 diagnosed with allergic rhinitis.

OBJECTIVES

General objective

1. To determine the diagnostic accuracy of the ALFA Specific Allergen Test compared to the skin prick test in detecting sensitization to house dust mite (*Dermatophagoides pteronyssinus*) in children aged 8 to 18 years old with allergic rhinitis seen at the Philippine General Hospital (PGH) Allergy Clinic.

Specific objective

1. To determine the sensitivity and specificity of the ALFA Specific Allergen Test in detecting sensitization to house dust mite (*Dermatophagoides pteronyssinus*) in children with allergic rhinitis.
2. To determine the ALFA Specific Allergen Test's positive and negative predictive values.
3. To determine the ALFA Specific Allergen Test's positive and negative likelihood ratios.
4. To identify the disadvantages of using the ALFA Specific Allergen Test in detecting house dust mites (*Dermatophagoides pteronyssinus*) in children ages 8 to 18 diagnosed with allergic rhinitis.

METHODOLOGY

Study design

This prospective study of diagnostic test accuracy was performed at the PGH Allergy Clinic.

Study population and setting

Inclusion criteria

a. Pediatric patients between the ages of 8 and 18 years diagnosed with allergic rhinitis who were seen at the PGH Allergy Clinic were recruited from September 2016 to March 2017.
b. Patients were taken off antihistamines, anxiolytics, systemic or topical corticosteroids for at least 1 week prior to enrollment in the study.

Exclusion criteria

a. Patients who have had a previous skin prick test done.
b. Patients receiving immunotherapy.
c. Patients without legally competent parents or guardians.

Sample size

This study included 77 patients based on available studies of allergic rhinitis, with a prevalence of 25%, a sensitivity of 95%, a specificity of 95%, a confidence level of 95%, and a precision of 10%. The minimum sample size computation was derived using the Excel template prepared by Naing L.[13]

Procedure

Informed consent was obtained from the parents or guardians by the primary investigator, and assent forms were obtained from the patients themselves. A detailed history and physical examination were obtained for each patient to assess whether they had symptoms of allergic rhinitis and to determine their eligibility for enrollment in the study.

The skin prick test against inhalant allergens was first performed by a Fellow-in-Training from the Section of Allergy and Immunology, blinded to the result of the Allergy Lateral Flow Assay (ALFA) Specific Allergen Test, on the volar surface of the patient's forearm after cleaning the area with alcohol. The skin prick test was performed in the area 3 cm from the antecubital fossa to 5 cm from the wrist. The skin prick test was done by placing a small drop of each test extract and control solutions. The drops were placed 2 cm apart. The skin was then pricked using a disposable lancet, which was passed through the drops and inserted into the epidermal surface. The results of the skin prick test were read after 15 to 20 minutes. Results of the skin prick test to house dust mite (*Dermatophagoides pteronyssinus*) were recorded. A positive result was defined as a wheal diameter of 3 mm or greater than the diluent negative control performed at the same time. A negative result was a wheal measurement of less than 3 mm compared to the negative control.

The Allergy Lateral Flow Assay (ALFA) Specific Allergen Test was then performed on the patient by the primary investigator to determine sensitization to house dust mite (*Dermatophagoides pteronyssinus*). The primary investigator was blinded to the results of the skin prick test. The reagent and cassette were brought to room temperature (18-25 °C) at least 30 minutes prior to the procedure. After bringing the cassette to room temperature, the cassette was unpacked and placed on a clean, stable table. The patient's fingertip was then wiped clean with an alcohol swab. The patient's fingertip was then pricked with a lancet, and the finger was massaged in the direction of the fingertip until a sufficient drop was produced. A drop of blood (~50 μL) was placed into the sample application point of the cassette. Whole blood was used for this test.

Immediately after transferring the blood into the cassette, two drops of the allergen solution (*Dermatophagoides pteronyssinus* extract) were placed into the sample

application field. The cassette was allowed to set for 30 minutes. After 30 minutes, the ALFA Specific Allergen Test was read by the primary investigator, blinded to the results of the skin prick test. A positive result produced a red line in both the test line (T) and the control line (C). A negative result produced a red line in the control line (C). A result that had no red line in both the test line (T) and the control line (C) was recorded as an invalid result.

Statistical analysis

Sensitivity and specificity of the Allergy Lateral Flow Assay (ALFA) Specific Allergen Test in detecting house dust mite (*Dermatophagoides pteronyssinus*) were computed, as well as the positive and negative predictive values. Likelihood Ratio for the ALFA Specific Allergen Test was also computed. Sensitivity and specificity were plotted on the Receiver Operating Characteristic (ROC) Curve, and the Area Under the Curve (AUC) was subsequently obtained.

Ethical considerations

The protocol was submitted, reviewed, and approved by the University of the Philippines Manila Research Ethics Board (UPMREB). The study was conducted after approval by the ethics review board. All patient information was anonymized and kept confidential. In participating in this research, there were no significant risks experienced from the ALFA Specific Allergen Test. Since the ALFA Specific Allergen Test involved pricking the participant's finger to obtain blood, minimal pain and discomfort were experienced by the participant.

Regarding the skin prick test, it is possible that the participant may experience adverse reactions to the extracts being tested. These reactions included wheals, rash, local itching, and rarely, difficulty of breathing. If these reactions occurred, the investigators administered emergency medications.

Through participation in this research, it was confirmed whether the participant had allergic rhinitis, and the triggers of their allergic rhinitis were identified, which aided in the control and management of their symptoms. The ALFA Specific Allergen Test and the Skin Prick Test were provided for free to the participant. There were no conflicts of interest for either the primary investigator or the super-vising investigators in the completion of this research.

Roles of members in the protocol

The primary investigator was responsible for recruiting patients for the study, obtaining informed consent from legally competent parents or guardians, and obtaining assent forms from the participants. The primary investigator performed the ALFA Specific Allergen Test, and a Fellow-in-Training performed the skin prick test from the Section of Allergy and Immunology, PGH. Dr. Roxanne Casis-Hao, Dr. Mary Anne R. Castor, Dr. Marysia Stella T. Recto, and Dr. Madeleine W. Sumpaico contributed to the design, analysis, and interpretation of the data, critically revised this manuscript for important intellectual content, and approved the final version.

RESULTS

Patients between the ages of 8 and 18 years were screened for any history and physical examination findings of allergic rhinitis seen at the PGH, Allergy Clinic, between September 2016 and February 2017. Seventy-seven patients were enrolled in this study, with 57% being male. The mean age of patients enrolled in this study was 8 years.

Both the skin prick test to house dust mite (*Dermatophagoides pteronyssinus*) and the ALFA Specific Allergen Test to house dust mite (*Dermatophagoides pteronyssinus*) were performed on each patient by separate investigators, who were blinded to the results of the other diagnostic tests. In this study, the sensitivity of the ALFA Specific Allergen Test to house dust mite (*Dermatophagoides pteronyssinus*) was computed to be 84.8% (95% CI 71.1% - 93.7%) while the computed specificity of the ALFA Specific Allergen Test to house dust mite (*Dermatophagoides pteronyssinus*) was 100% (95% CI 88.8%-100%). The ALFA Specific Allergen Test has 100% (95% CI 91%-100%) and 81.6% (95% CI 65.7%-92.3%) positive and negative predictive values, respectively. The positive likelihood ratio of the ALFA Specific Allergen Test could not be computed since specificity was 100%; the negative likelihood ratio of the ALFA Specific Allergen Test was 0.152 (Table 1).

The AUC was also calculated to be 92.4% (95% CI 87.1%-97.6%) as shown below (Figure 1).

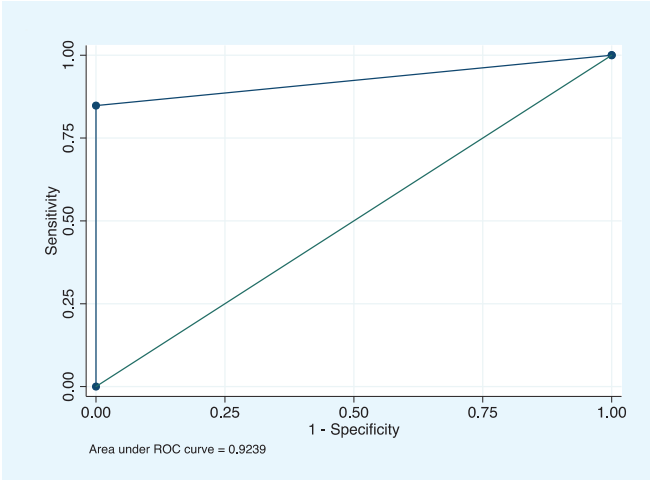

Figure 1. Results of the ALFA specific allergen test plotted on the receiver operating characteristic (ROC) curve.

Table 1. Tabulated results of the statistical analysis

	ALFA Specific Allergen Test
Sensitivity	84.8% (95% CI 71.1% - 93.7%)
Specificity	100% (95% CI 88.8%-100%)
Receiver Operating Curve (ROC)	0.924 (95% CI 87.1%-97.6%)
Likelihood ratio (+)	~
Likelihood ratio (-)	0.15
Positive Predictive Value	100% (95% CI 91%-100%)
Negative Predictive Value	81.6% (95% CI 65.7%-92.3%)

DISCUSSION

The hallmark of any true allergic condition, such as allergic rhinitis, is an evidence of specific IgE to a relevant allergen in the affected individual. Skin prick tests are widely used to demonstrate an immediate IgE-mediated allergic reaction. They represent a major diagnostic tool in the field of allergy because they are easy to perform, inexpensive, and provide a positive or negative response within a few minutes.

If the skin prick test cannot be performed, multiple *in vitro* alternative tests have been developed to determine the specific IgE levels against allergens. These tests measure specific IgE antibodies and are an important complementary tool for diagnosing allergies, especially in patients with extensive eczema, dermatographism, urticaria, or those who cannot be taken off antihistamines. The Allergy Lateral Flow Assay (ALFA) Specific Allergen Test is a new point-of-care specific IgE test that provides a rapid assay for the qualitative determination of allergen-specific IgE in human sera, plasma, or whole blood, producing results in 30 minutes.

In this study, the diagnostic accuracy of the ALFA Specific Allergen Test was evaluated in children aged 8 to 18 years with a diagnosis of allergic rhinitis, who were seen at the PGH Allergy Clinic. The results of the study revealed that the ALFA Specific Allergen Test had a sensitivity of 84.8%. This means that 84.8% of patients with the presence of specific IgE to house dust mite (*Dermatophagoides pteronyssinus*) based on the skin prick test were correctly classified as having specific IgE to house dust mite (*Dermatophagoides pteronyssinus*) based on the ALFA Specific Allergen Test. Hence, around 15% of patients with specific IgE, as determined by skin test, had negative ALFA Specific Allergen Test results (false negatives). The ALFA Specific Allergen Test is a highly specific test with a computed specificity of 100%. This means that all patients with a negative skin prick test to house dust mite (*Dermatophagoides pteronyssinus*) were correctly classified as having no specific IgE to house dust mite (*Dermatophagoides pteronyssinus*) based on the ALFA Specific Allergen Test.

The ALFA Specific Allergen Test had a 100% positive predictive value, indicating that this test can detect 100% of patients with specific IgE levels to house dust mite (*Dermatophagoides pteronyssinus*). This means that if the ALFA Specific Allergen Test is positive, one can be 100% sure that the patient is sensitized to house dust mite (*Dermatophagoides pteronyssinus*). The ALFA Specific Allergen Test had a negative predictive value of 81.6%, which means that with a negative ALFA Specific Allergen Test result, around 81.6% of them will have absent specific IgE to house dust mite (*Dermatophagoides pteronyssinus*). So, if the test is negative, one is 81.6% sure that the patient does not have specific IgE to house dust mite (*Dermatophagoides pteronyssinus*). Hence, a positive test would be more useful.

The positive Likelihood Ratio for the ALFA Specific Allergen Test to house dust mite (*Dermatophagoides pteronyssinus*) cannot be computed since the computed specificity was 100%. The negative Likelihood Ratio for the ALFA Specific Allergen Test to house dust mite (*Dermatophagoides pteronyssinus*), on the other hand, was 0.15. This means that the probability of getting a negative result of the ALFA Specific Allergen Test among the patients who have specific IgE antibody is 85% lower compared to the probability of getting a negative result of the ALFA Specific Allergen Test to house dust mite (*Dermatophagoides pteronyssinus*) among patients who truly do not have the presence of specific IgE antibody to house dust mite (*Dermatophagoides pteronyssinus*).

The specificity and sensitivity computed for the ALFA Specific Allergen Test to house dust mite (*Dermatophagoides pteronyssinus*) were also plotted on the Receiver Operating Characteristic (ROC) Curve. ROC analysis is used in clinical epidemiology to quantify how accurately medical diagnostic tests (or systems) can discriminate between two patient states, typically referred to as "diseased" and "non-diseased". The ROC is a plot of the true positive rate (sensitivity) against the false positive rate (1-specificity). Any increase in the sensitivity would result in a decrease in the specificity. The closer the curve is to the left-hand border and the top border, the more accurate the test is.[14] Upon plotting, it was noted that the curve was closer to the left-hand border and the top border of the ROC Curve, which means that the results of the ALFA Specific Allergen Test to house dust mite (*Dermatophagoides pteronyssinus*), based on the ROC Curve, are close to accurate. Test accuracy is measured by the area under the curve (AUC). An area of 1 is a perfect test, while an area of 0.5 is a worthless test.[14] The AUC was 0.924. This means that 92.4% of patients sensitized to house dust mite (*Dermatophagoides pteronyssinus*) would be correctly classified as sensitized using the ALFA Specific Allergen Test. This shows that the ALFA Specific Allergen Test to house dust mite

(*Dermatophagoides pteronyssinus*) is a reliable test in determining the presence and absence of specific IgE to house dust mite (*Dermatophagoides pteronyssinus*).

Since the ALFA Specific Allergen Test for house dust mite (*Dermatophagoides pteronyssinus*) yielded a sensitivity of 84.8% and a specificity of 100%, these results were then compared to those of the Pharmacia ImmunoCAP, a more extensively studied *in vitro* test, which yielded a sensitivity of 96.2% and a specificity of 90.9% to *Dermatophagoides pteronyssinus*.[15] Between the 2 *in vitro* assays, the Pharmacia ImmunoCAP is more useful for screening patients suspected of having sensitization to house dust mite (*Dermatophagoides pteronyssinus*). However, the ALFA Specific Allergen Test for house dust mite (*Dermatophagoides pteronyssinus*) is more specific compared to the Pharmacia ImmunoCAP. Hence, it is more useful in confirming the absence of sensitization to house dust mite (*Dermatophagoides pteronyssinus*).

The ALFA Specific Allergen Test also has some advantages over the Pharmacia ImmunoCAP, in relation to the procedure. This test can be performed in a clinic setting, with results produced in 30 minutes, compared to the Pharmacia ImmunoCAP, which takes around 1 day. The ALFA Specific Allergen Test is more cost-effective than the Pharmacia ImmunoCAP. It costs PhP 1,200.00 per allergen while the Pharmacia ImmunoCAP costs PhP 2,100.00 per allergen. However, the skin prick test is still the most cost-effective compared to the ALFA Specific Allergen Test and the Pharmacia ImmunoCAP. The skin prick test costs around PhP 115.00 per allergen in the private clinics and PhP 70.00 per allergen at the Philippine General Hospital Allergy Clinic.

The drawback noted for the ALFA Specific Allergen Test is that this *in vitro* assay utilizes human sera, plasma, or whole blood to determine the presence of specific IgE. If one allergen were tested on a patient, a single prick on the finger would be enough to produce a positive or negative result, and this test can be performed with ease in the clinic setting. However, if a physician would like to determine specific IgE on multiple allergens, more serum or whole blood will be required for the test to be performed. Therefore, the ALFA Specific Allergen Test can only test a certain number of multiple allergens at a time. Testing for several allergens would require more blood specimens, and the cost would escalate. Furthermore, the allergen extracts currently available for this test are not all applicable in the Philippines, particularly with the pollen extracts, since the Philippines has a different flora compared to Western countries.

CONCLUSION

In this study, the detection of sensitization to house dust mite (*Dermatophagoides pteronyssinus*) using the ALFA Specific Allergen Test was shown to be moderately sensitive and highly specific, using the skin prick test as the reference standard. Upon plotting the sensitivity and specificity on the ROC Curve, it was seen that the ALFA Specific Allergen Test is a reliable test in determining the presence and absence of specific IgE to house dust mite (*Dermatophagoides pteronyssinus*). It is more cost-effective than the Pharmacia ImmunoCAP and is a rapid *in vitro* test that can be used to determine the presence of specific IgE to house dust mite (*Dermatophagoides pteronyssinus*).

It is recommended that further studies with other allergens be performed to see if the ALFA Specific Allergen Test can be used as an alternative test for the allergy skin prick test in patients for whom the allergy skin prick test cannot be done.

Statement of Authorship
All authors certified fulfilment of ICMJE authorship criteria.

Author Disclosure
The authors declared no conflict of interest.

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